

CLAIMS

1. A method of amplifying a target RNA containing a specific base sequence in a sample by an RNA amplification procedure which comprises a step of forming  
5 a double-stranded DNA which has a promoter sequence and is capable of being transcribed into an RNA consisting of the specific base sequence or a sequence complementary to the specific base sequence by using the target RNA as the template, a step of forming an RNA transcript consisting  
10 of the specific base sequence or a sequence complementary to the specific base sequence by using an RNA polymerase and a step of forming the double-stranded DNA by using the RNA transcript as the template, in the presence of adenosine triphosphate, uridine triphosphate, cytidine  
15 triphosphate, guanosine triphosphate and inosine triphosphate as the substrates of the RNA polymerase.
2. The amplification method according to Claim 1, wherein RNA polymerase from phage SP6 is used as the RNA polymerase, and inosine triphosphate is added to a final  
20 concentration of from 0.5 mM to 2 mM in the amplification procedure.
3. The amplification method according to Claim 2, wherein the ratio of the final concentration of inosine triphosphate to the final concentration of the other  
25 ribonucleoside triphosphates (adenosine triphosphate, uridine triphosphate, cytidine triphosphate and guanosine triphosphate) is from 0.5:1.0 to 1.5:1.0.

4. The amplification method according to Claim 1,  
wherein in the amplification procedure, tris-HCl buffer  
(pH 8.5-8.9) is present at a final concentration of from  
20 mM to 50 mM, magnesium chloride is present at a final  
5 concentration of from 12 mM to 20 mM, ribonucleoside  
triphosphates (adenosine triphosphate, uridine  
triphosphate, cytidine triphosphate and guanosine  
triphosphate) are present at a final concentration of 3.5  
mM to 5.0 mM, RNA polymerase from phase T7 is present as  
10 the RNA polymerase, and inosine triphosphate is present  
at a final concentration of from 1.0 mM to 2.7 mM.

5. The amplification method according to Claim 4,  
wherein the ratio of the final concentration of inosine  
triphosphate to the final concentration of the other  
15 ribonucleoside triphosphates (adenosine triphosphate,  
uridine triphosphate, cytidine triphosphate and guanosine  
triphosphate) is from 0.3:1.0 to 0.7:1.0.

6. The amplification method according to Claim 1,  
wherein in the amplification procedure, tris-HCl buffer  
20 (pH 8.5-8.9) is present at a final concentration of from  
50 mM to 80 mM, magnesium chloride is present at a final  
concentration of from 12 mM to 20 mM, ribonucleoside  
triphosphates (adenosine triphosphate, uridine  
triphosphate, cytidine triphosphate and guanosine  
25 triphosphate) are present at a final concentration of 2  
mM to 3.5 mM, RNA polymerase from phage T7 is present as  
the RNA polymerase, and inosine triphosphate is present

at a final concentration of from 3.2 mM to 4.4 mM.

7. The amplification method according to Claim 6,  
wherein the ratio of the final concentration of inosine  
triphosphate to the final concentration of the other  
5 ribonucleoside triphosphates (adenosine triphosphate,  
uridine triphosphate, cytidine triphosphate and guanosine  
triphosphate) is from 1.0:1.0 to 1.0:1.5.

8. The amplification method according to Claim 1,  
wherein the RNA amplification procedure uses a primer  
10 complementary to the specific base sequence and a primer  
homologous to the specific base sequence (either of which  
is a promoter primer having a promoter sequence for the  
RNA polymerase at the 5' end) and is characterized in  
that the target RNA is used as the template to form a  
15 single-stranded DNA by the action of an RNA-dependent DNA  
polymerase, the single-stranded DNA is used as the  
template for formation of a double-stranded DNA which has  
a promoter sequence and is capable of being transcribed  
into an RNA having the specific base sequence or a  
20 sequence complementary to the specific base sequence by  
the action of a DNA-dependent DNA polymerase, the double-  
stranded DNA is transcribed into an RNA transcript in the  
presence of the RNA polymerase, and the RNA transcript is  
used as the template for the subsequent formation of a  
25 single-stranded DNA by the RNA-dependent DNA polymerase.

9. A method of assay of a target nucleic acid which  
comprises carrying out the amplification procedure as

defined in Claim 1 in the presence of a probe labeled with a fluorescent intercalative dye, and monitoring the fluorescence intensity of the reaction solution.

10. The method according to Claim 9, wherein the probe  
5 labeled with a fluorescent intercalative dye alters its fluorescence upon hybridization with the RNA transcript.